THE MICROBIOME, AUTOIMMUNITY, AND ARTHRITIS: CAUSE AND EFFECT

AN HISTORICAL PERSPECTIVE

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The etiology of arthritis has been sought for centuries, employing the art and science of the times to clarify, identify, and establish a cause and a cure. None other than Sir William Osler in his 1909 edition of *Principles and Practice of Medicine* stated that the etiology/origin of rheumatoid arthritis was related to "the nervous system or infections, with exciting causes that included age, gender, family history, cold, damp, errors in diet, worry and care, and local injuries." Since Osler's observation more than 100 years ago, there has been tantalizing, but often inconclusive, evidence about the etiological role that microorganisms play in the development of arthritis and autoimmune diseases. Current therapy focuses on the pathogenesis rather than the etiology of these disorders. To rein in the overactive immune system we believe is causing the disease, we use immunosuppressive drugs, an act that would be counterintuitive if infection were the root cause of the problem.

We have come light-years from Osler's clinical observations with regard to our understanding of the innate and adaptive immune systems and how they interact, the interplay of the nervous system and aspects of the immune system, appreciation of the genome and microbiome of humans and microorganisms, recognition of the impact of trauma as well as environmental and nutritional factors upon health. Despite this increased understanding, we have still not defined the etiology of many types of tissue- and life-altering arthritis and autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, scleroderma, the vasculitides, psoriatic arthritis, and ankylosing spondylitis.

We are closer, however, to understanding the connection between micro-organisms and reactive arthritis, and we have come much far-

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ther in establishing a connection between infectious agents and septic arthritis, Lyme disease and *Borrelia burgdorferi*, mixed cryoglobulinemia and hepatitis C, polyarteritis nodosa and hepatitis B, group A beta-hemolytic streptococci and rheumatic fever, and Whipple's disease and *Tropheryma whipplei*. It is these disorders that will be the subject this paper.

HISTORY OF INFECTIONS

The account of the acquisition of knowledge of infectious organisms and the infectious diseases they cause has been one of the wonders of scientific exploration since plagues were first described in detail between 1000 and 1380 AD (1). Important milestones (Table 1) include the development of the microscope in 1600 by Antoni van Leeuwenhoek; John Snow and his innovative hypothesis that cholera was an infectious disease spread by fecal contamination of drinking water, and the modern era of epidemiologic research that it launched; the development of statistics and surveillance in the 19th century including William Farr's use of numerator and denominator data in the calculation of the standardized mortality rate; the discovery of microorganisms and the development of vaccines; antiviral drugs; antibiotics; and one of the first uses of modern experimental study design in defining the efficacy of streptomycin in the treatment of pulmonary tuberculosis in the late 1940s.

After Louis Pasteur and Robert Koch redefined modern microbiology in the late 1800s, Koch and Loeffler postulated four basic prerequisites for proof that a micro-organism causes disease. These included demonstration that the organism is isolated in every case of the disease; the ability to cultivate the organism in pure culture; evidence that the

TABLE 1
History of Infections

Year	Event
1000–1300	Plagues
400–1500	Early epidemiology
1600	Observation and care of the patient; the microscope
1813	John Snow: modern epidemiology
1600–1800	Development of statistics and surveillance
1600–1900	Discovery of micro-organisms
1882–1895	Pasteur: fermentation and micro-organisms
1843–1910	Koch: anthrax, TB, cholera
1798-current	Vaccines, antibiotics, antivirals
Current	HIV, Lyme, prions, bacterial resistance, microbiome, molecular genetics

cultured organisms could induce the disease in experimental animals; and the capacity to recover the organism from the infected experimental animals. From the onset, these postulates lacked a universal application because many bacteria and viruses were found in asymptomatic individuals, not all subjects exposed will acquire an infection due to the importance of host factors such as genetics, and advances in modern nucleic acid–based microbiologic detection methods made the original postulates even less relevant. The modernized 1996 Fredericks and Relman criteria incorporated more contemporary scientific constructs and demanded that a nucleic acid sequence should be present; fewer, or no, copies of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease; and with resolution of the disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable (2).

THE MICROBIOME

In 2002, Nobel laureate Joshua Lederberg coined the terms superorganism and microbiome to describe the ensemble of human and non-human (micro-organism) cells that constitute our body. He noted that intestinal microbiota are able to shape the immune system to maintain homeostasis and healthy states or promote inflammation when the composition of the microbiota community becomes imbalanced, a state termed dysbiosis (3).

In 2007, the National Institutes of Health launched the Human Microbiome Project to characterize the microbial communities found at several different sites in the human body, including nasal passages, oral cavities, skin, gastrointestinal tract, and urogenital tract, and to analyze the role of these microbes in human health and disease. Metagenomic analyses were used to determine whether there is a core microbiome at each site, one that could be associated with specific disease states. This initiative began with the sequencing of genomes from both cultured and uncultured bacteria, plus several non-bacterial microbes. Using advances in sequencing technologies, it became clear that more than three quarters of the bacteria living in our bodies had never been cultured because the growth of these micro-organisms were not favored by the nutrients and conditions commonly used in our microbiology laboratories. Thus, micro-organisms can be defined now by culture-independent DNA sequencing to perform taxonomic identification and to characterize enzymatic function of bacteria. This is a profound paradigm shift wherein bacteria are characterized more by their unique molecular fingerprints than by their behavior on an agar plate (4).

HUMAN ORGAN-SPECIFIC AUTOIMMUNE DISEASES WITH MICROBIAL TRIGGERS

Inflammatory joint diseases and autoimmune disorders have been hypothesized as conditions that are likely triggered or exacerbated by micro-organisms because of the fact that phlogistic/immunologically generated symptoms and signs of infectious disorders such as tuberculosis, syphilis, and Lyme disease are shared by autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, and vasculitides such as polyarteritis nodosa and granulomatosis with polyangiitis (Wegener's granulomatosis). These shared symptoms of fever, fatigue, weight loss, joint inflammation, neurologic disorders, and skin lesions likely reflect the common immunological mechanisms in both, such as the liberation of the pro-inflammatory cytokines tumor necrosis factor alpha and interleukins 1 and 6, and activation of both the innate and adaptive arms of the immune system. That the etiology of the infectious scourges of the past centuries was defined, and safe and effective antibiotics and anti-viral agents developed, gives hope to the patients who live with autoimmune diseases and an extra stimulus to the scientists investigating them.

Table 2 lists some disorders in which there are cross-reacting immunological factors that connect a micro-organism with a disease. Moreover, not only can we demonstrate the recognition of a specific micro-organism by the immune system, but these disorders can be treated with or prevented by antibiotics and antiviral agents, the later in combination with immune manipulation. Lyme disease is responsive to antibiotics such as doxycycline; suppression of group B beta hemolytic streptococci using penicillin can prevent rheumatic fever recurrence; polyarteritis nodosa triggered by hepatitis B can be successfully controlled with a combination of antiviral agents, plasmapheresis, and a short course of corticosteroids; Whipple's disease is treatable with antibiotics; and the systemic disease associated with

TABLE 2
Human Organ-specific Autoimmune Diseases With Its Microbial Trigger (5)

Disease	Organism	Immunologic Factor
Rheumatic heart disease	Streptococci	T cell response to strep M5 protein
Guillain-Barre	Campylobacter	Cross reacting Ab to gangliosides
Polyarteritis nodosa	Hepatitis B	Immune complexes
Lyme arthritis	Borrelia bergdorferi	LFA-1 mimicry of OspA; T cell response to OspA
Chronic autoimmune hepatitis	Hepatitis C	Epitopes differ from autoimmune hepatitis
Myocarditis	Coxsackie virus B3	Response to cardiac myosin

hepatitis C–triggered mixed cryoglobulinemia is controlled with antiviral drugs (5).

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a systemic autoimmune disease manifested by a symmetrical polyarthritis of the small joints of the hands and feet that can lead to progressive joint inflammation and damage unless treated aggressively and in its earliest stirrings. Over the years, both epidemiologic and clinical research has implicated micro-organisms such as mycoplasma and Epstein-Barr virus in the pathogenesis of rheumatoid arthritis. However, no cause and effect relationship has been defined and, although antibiotics such as tetracycline have been shown to have a mild ameliorative clinical effect on rheumatoid arthritis perhaps related to inhibition of metalloproteinases, most of the therapy today is aimed at damping down an over-exuberant immune system rather than focusing on an infectious etiology. Over the past century, the concept of an association between rheumatoid arthritis and intestinal or oral micro-organisms has been put forward but never definitively proven. Recent evidence has suggested that distinctive enterotypes may exist in RA and that periodontopathic bacteria might be a link between RA joint inflammation, peptide citrullination with the development of autoantibodies and periodontitis. How such intriguing data will eventuate in the identification of potential therapeutic targets, the discovery of unique biomarkers, or even preventative approaches, remains to be seen (6, 7).

WHIPPLE'S DISEASE

Whipple's disease is a rare, chronic, complex systemic disorder that is caused by a bowel infection from the Actinomycetes-like organism *Tropheryma whippeli* and is antibiotic responsive. Table 3 lists the clinical features of Whipple's disease along with other disorders that can present in a similar clinical fashion, supporting the broad differential diagnosis that must be considered before contemplating the possibility of Whipple's disease and then defining it by appropriate tissue biopsy, histological staining, and polymerase chain reaction testing. This illness can exist for decades before the development of the telltale gastrointestinal and malabsorption symptoms and signs and the ability of the organism to co-exist with the host and live within its macrophages seems to be related to both macrophage and T cell defects, not humoral ones.

To the medical historian in all of us, a reading of the milestones in the history of Whipple's disease is as exciting and interesting as

TABLE 3
Whipple's Disease

Clinical Features	Differential Diagnosis
Male gender (87%)	
Arthralgias/-itis (73%)	SLE/rheumatoid arthritis
Diarrhea (81%)	Inflammatory bowel disease
Weight loss (95%)	Malignancy
Fever (38%)	SBE
Adenopathy (52%)	Sarcoidosis/lymphoma
Melanoderma (41%)	Endocrinopathy
Neurologic sign (33%)	Multiple sclerosis
Pleura effusion (14%)	Tuberculosis
Ocular disease (6%)	Behcet disease

reading a Sherlock Holmes story written by Sir Arthur Conan Doyle. It begins at Johns Hopkins Hospital in 1907 with the description by George Hoyt Whipple of a disease in a 36-year-old physician that was characterized by steatorrhea, weakness, loss of weight, anemia, polyarthritis, and cough. At post-mortem, the most striking finding was a massive deposition of fat in the intestinal and mesenteric lymphatic tissues leading Whipple to name this disease "intestinal lipodystrophy." Technological advances over the next 100 years enabled investigators to display PAS-positive rods in lamina propria macrophages, define these rods as bacteria using the electron microscope by this association's Thomas Hendrix et al, treat the disease with antibiotics, perform16S rRNA sequencing of *T. whippeli*, cultivate the organism, and eventually fully sequence the genomes from different strains of *T. whippeli* (8, 9).

LYME DISEASE

Lyme disease is the most common tick-borne disease in the Northern Hemisphere. The infectious trigger *Borrelia burdorferi* is transmitted to humans by the bite of infected ticks belonging to a few species of the genus *Ixodes*. Early symptoms may include fever, headache, and a characteristic circular skin rash called erythema migrans. Left untreated, later symptoms may involve the joints, heart, and central nervous system. In most cases, the infection and its symptoms are eliminated by antibiotics, especially if the illness is treated early. Delayed or inadequate treatment can lead to the more serious symptoms, which can be disabling and difficult to treat.

The clear and simple description above does not give adequate credit to the extraordinary scientific journey initiated by Allan Steere and his colleagues at Yale University in 1977. It started in three Connecticut

communities east of the Connecticut River where multiple cases of oligo-articular arthritis in children and adults occurred. Initially, it was thought to be an outbreak of juvenile rheumatoid arthritis. Epidemiological evidence soon allowed the connections to be made between a tick vector, the *Borrelia burdorferi* spirochete, the characteristic erythema chronicum migrans rash, and the clinical manifestations of Lyme disease and Lyme arthritis. By 1984, Lyme disease was defined as a unique human model for an infectious etiology of rheumatic disease. Eventually, the use of parenteral penicillin successfully treated patients with established Lyme arthritis, an animal model for Lyme disease was developed, sensitive and specific serodiagnosis was established using both ELISA assays and immunoblotting, and the treatment of both early and antibiotic refractory Lyme arthritis was defined and included either oral or parenteral antibiotics.

There has been support for an autoimmune component of antibiotic-refractory Lyme arthritis in the form of its association with the class II histocompatibility antigens HLA-DR4 and HLA-DR2 (HLA associations shared with rheumatoid arthritis), T-cell recognition of *Borrelia burgdorferi* outer surface protein 163-175, and the finding of persistent synovial inflammation after the near or total eradication of spirochetes. Thus, anti-inflammatory drugs and even disease-modifying drugs used for rheumatoid arthritis such as hydroxychloroquine are used for patients in whom the Infectious Disease Society of America's recommended antibiotic courses are not successful. Again, we see the nexus between well-defined infectious disorders and chronic inflammatory disorders (10–12).

REACTIVE ARTHRITIS

The term reactive in this context is meant to represent an immune response of the body to an organism with a subsequent cross-reaction to host tissue through the immunological process of molecular mimicry. Molecular mimicry is defined as the theoretical possibility that sequence similarities between foreign and self-peptides are sufficient to result in the cross-activation of autoreactive T or B cells by pathogen-derived peptides. This can take various clinical forms including rheumatic fever due to group A beta hemolytic strepococci that can cause migratory arthritis, heart and brain damage, and a non-septic reactive arthritis that follows an extra-articular infection of the genitourinary or gastrointestinal tract and is either self-limited or lifelong with the potential to cause destructive peripheral joint, spine, and eye inflammation. The organisms that were found to be triggers included

Salmonella, Shigella, Campylobacter, *Clostridium diffficile*, and Yersinia in the GI tract and Chlamydia in the genitourinary tract (13).

In 1966, Zabriskie et al at the Rockefeller University demonstrated cross-reactive antibodies to the M protein of the streptococcal cell wall and cardiac myosin. This groundbreaking work gave credence to the theory of molecular mimicry and triggered a number of investigations that addressed the possibility that molecular mimicry was the basis for reactive arthritis (14, 15). In 1973, an association was made between ankylosing spondylitis and a class I histocompatibility antigen HLA-B27. Studies then demonstrated that HLA-B27 was also a risk factor for reactive arthritis. Soon, investigations showed a sequence homology between B27 and arthritogenic organisms (16). Monoclonal antibodies and T-cell clones were found to react with both HLA-B27 and the putative inciting organism (17). Eventually, single nucleotide polymorphisms in Toll-like receptor (TLR) 2 were found to confer susceptibility to reactive arthritis due to Salmonella (18). Thus, inflammation triggered by an infection required neither a viable pathogen nor an intact organism. The arthritis, dermatitis, and enteritis found in B27 transgenic rats were ameliorated if the animal was raised in an infection-free environment. Re-establishment of the clinical features of that model did not require a gut infection in the usual sense. Rather, a common gut commensal Bacteroides, not previously thought to be an arthritogenic pathogen, could re-establish the clinical features in that model (19, 20). Thus, a gut population with commensal organisms can lead to an immunological platform on which arthritis can proceed. In the polyarthritis IL-1-Ra deficient mouse model, re-establishment of arthritis in a germ-free setting can be triggered by TLR signaling alone.

The recognition that Chlamydia species might exist in a persistent metabolically active infection state in the synovium of patients with reactive arthritis suggested that they may be susceptible to prolonged courses of particular antibiotics that addressed particular biologic characteristics of the organism and its life cycle. A recent 9-month, double-blind, prospective trial assessing a 6-month course of combination antibiotics as a treatment for Chlamydia-induced reactive arthritis revealed that antibiotic treatment (doxycycline + rifampin or azithromycin + rifampin) was associated with significant improvement in joint inflammation. As Robert Inman, MD, one of the authors of this landmark study, stated so eloquently, “This study has raised important issues in several contexts. First, there is a conceptual issue that intra-articular persistence of pathogens may trigger a chronic inflammatory process that may be sustained, possibly for years. Secondly, the

ambiguity in nomenclature becomes readily apparent, as it has been the case for Lyme arthritis. If the inflammatory process is terminated with antibiotic treatment, it could be argued that the treated condition meets a reasonable operational definition of septic arthritis, rather than reactive arthritis. Finally, the clinical implications are significant. The subjects of the study noted above had had a long-standing seronegative arthritis that had proved refractory to both nonsteroidal anti-inflammatory drugs and disease modifying antirheumatic drug treatment. This raises a very pertinent clinical question: How many patients currently classified as undifferentiated spondyloarthropathy might be effectively treated with antibiotics?"(16)

PERSPECTIVE

As our understanding of the microbiome, the genome, and the immune system becomes clearer, we will likely find the interface between them blurring as we discover that increasing numbers of so-called "idiopathic" systemic disorders such as lupus and rheumatoid arthritis are either caused or modulated by micro-organisms, some of which are currently either unknown to us or misunderstood regarding their potential for causing human disease. Paradigms have and will shift as knowledge expands.

We have previously believed that the immune response to infection is overactive in non-septic disorders such as reactive arthritis. However, host responses may actually be diminished in view of data showing down-regulation of pro-inflammatory cytokines in experimental reactive arthritis, a state that could lead to an impaired ability of the host to clear organisms and thus its persistence. In the end: Should we be enhancing immunity as opposed to suppressing it (21)? The answer to this question and achievement of the optimal therapeutic balance hangs on our definition of the etiology of these disorders through continued research.

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DISCUSSION

Mackowiak, Baltimore: Superb talk, on time, Steve. Congratulations. The idea that rheumatoid arthritis is a new world disease is fascinating. I doubt if many people have heard of that concept. I've just barely heard about it and I'd be interested in any more information you could give us on that and why we believe that to be the case, and if you have any ideas of how that might relate to your microbiome theory.

Paget, New York: Yes. It's a very good question, and the fact is, when you look back in history, you can see a very clear demonstrations of gout and osteoarthritis, but you don't see rheumatoid arthritis, which has a telltale pathological and bony abnormality. What I think the question really highlights is that there have been migrations over time, and during them, certain genetically predisposed people have probably come upon new organisms, and that interaction has probably led to this disease.

Autwaerter, Baltimore: Fascinating, wonderful. I always wondered if the reactive arthritides and Lyme disease, will generally run their courses over 5 or 6 years, even in those antibiotic refractory patients. Is it your sense from looking at many of these other examples that there might be antigenic drivers, or is this something that is immunologically set in some other way that then diminishes over time because this obviously seems to be a limited process mostly?

Paget, New York: It seems to be very much related to the host and its interaction with the organism, and I think that can vary tremendously in all our diseases. The spectrum is quite amazing. You can basically put a given person's name on their illness with regard to how it evolves and its responsiveness to therapy. So, my feeling is that the host factors are quite key as well as other potential superimposed factors such as immunological deficiencies or other organisms that are infecting them at the same time.

Quesenberry, Providence: We see a smattering of babesia infections after Lyme disease, and recently I saw one where we thought it was a case of ITP and smears were negative, and all of a sudden serologies became positive as we persisted in considering this diagnosis. The patient responded dramatically to treatment. I am just wondering what you know about chronic babesia in this setting?

Paget, New York: Well, the same ticks can transmit these different types of diseases at the same time. As a matter of fact, some of these people who do not respond quickly to Lyme disease that has been defined by serologies may very well have superimposed organisms that you then have to treat in an alternative way. The phenotype could be very different than the usual and it could be explained by the superimposition of two different infections in the same host at the same time.

Quesenberry, Providence: Is it that like 5% to 10% of Lyme will have coexistent babesia.

Paget, New York: That's correct. Exactly.